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PRELIMINARY NOTES ON DISTOMUM PATELLARE, n. sp.

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FOR the material used in making this description I am indebted to Dr. Whitman, by whom this Distomum was found in the bladder of Triturus (Molge) pyrrhogaster Boie of Japan. The specific name was suggested to Dr. Whitman by Professor Leuckart.

The total length averages in the living specimen 4.5 mm., and of this the length of the neck forms about two-fifths. The body, which is nearly circular, averages 3 mm. at its widest part and is .4 mm. thick. The ventral sucker is a trifle larger than the oral sucker and averages .65 mm. in width.

Anatomical. — The cuticle has no spines, but is covered with small wartlike thickenings among which papillae more conspicuous in size and shape, the sensory papillae, are irregularly scattered. The sensory papillae average 50 μ apart on the ventral surface and somewhat more on the dorsal, and are largest around and within the borders of the suckers, the body papillae projecting 5 μ, those of the suckers 10 μ, above the level of the cuticle. The cuticle lines the external openings of the various ducts, ceasing where their epithelium begins. Laurer's duct and the excretory vesicle, which have no epithelium, are lined throughout by a thin membrane which is con-

tinuous with the cuticle, but lacks its wartlike thickenings. Underlying the cuticle is the subcuticula, which consists of

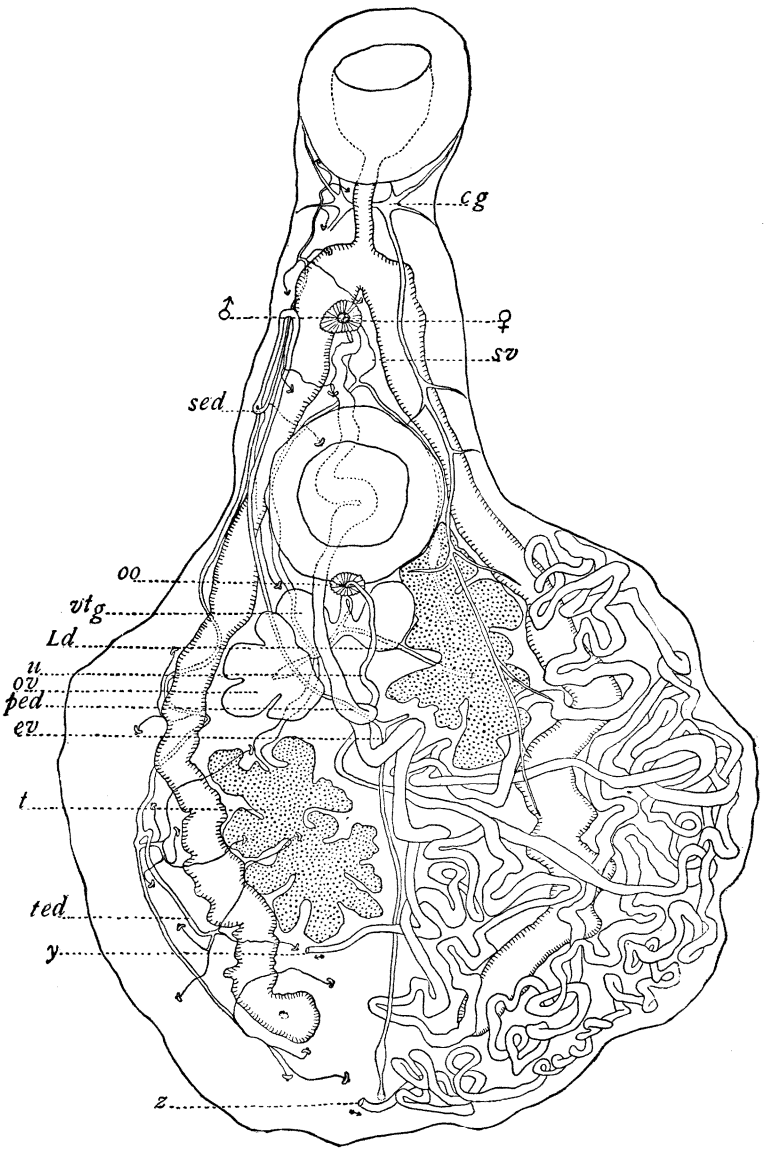

FIG. 1. — Ventral view. *cg*, cerebral ganglion; *ev*, excretory vesicle; *Ld*, Laurer's duct; *oo*, ootype; *ov*, ovary; *ped*, primary excretory duct; *sed*, secondary excretory duct; *sv*, seminal vesicle; *t*, testis; *ted*, tertiary excretory duct; *u*, uterus; *vtg*, vitelline gland; *y–z*, uterus upon right side, omitted in figure.

an external limiting membrane into which muscle fibers are inserted, and an internal non-cellular reticulum in which the dermal muscles are embedded. A continuation of the subcuticula sheaths the inner surfaces of the suckers. The dermal musculature shows the usual arrangement, — outer circular, middle longitudinal, and inner diagonal fibers. The oral sucker shows its adaptation to food-taking by the especial development of sphincter fibers, while its extrinsic muscles are weak ; the ventral sucker has few sphincters, but its base is fairly sheathed in a powerful extrinsic musculature of protractors and retractors, which pass in various directions from its sides to the body wall, so that the sucker could readily be shifted in position. The cellular structure of the parenchyma is distinct. The peripheral or subcuticular cells are smaller and more deeply staining than the rest, which look in comparison pale and vacuolar. The subcuticular cells are most conspicuous in the neck, but are coextensive with the cuticle. In the suckers they are also scattered among the vacuolar parenchyma cells.

D. patellare resembles in internal anatomy (Fig. 1) *D. folium*, which is described by Looss [1 (a)] as occurring in the bladder of certain fishes. The digestive tract has no salivary glands and no pharynx, although the thickened cuticle and musculature of the oesophagus may present traces of a pharynx. The intestinal epithelium has long abundant cilia. Each cerebral ganglion gives rise to two anterior and two posterior nerves, all of which give off branches which pass toward the body wall. No commissures were seen between the main trunks or their branches. The excretory system resembles more closely than that of *D. folium* that of *D. cygnoides* which occurs in the bladder of the frog and which Looss [1 (a)] considers closely related to *D. folium.* The excretory vesicle, which is much elongated and has a slight fusiform expansion in front of the excretory opening, has no epithelium, but the walls of the excretory ducts from the point where they leave the vesicle

[1] Looss, A.: (a) Die Distomen unserer Fische und Frösche, *Bibliotheca Zoologica*, Leuckart u. Chun, 1894.

(b) Zur Frage nach der Natur des Körperparenchyms bei den Trematoden, *Abhand. sächs. Gesell. d. Wiss.*, 1893.

consist of a distinct epithelium. The capillaries are not usually given off from short secondary or tertiary ducts as in *D. cygnoides*, but leave the primary or secondary trunks directly, so they are often of considerable length. The number of flame-cells varies and is seldom the same on both sides; there are usually from twenty-five to thirty on a side. The excretory ducts and capillaries are convoluted, owing probably to body contraction. The ovary lies, in more than half the specimens studied, upon the right side, in the rest of the specimens upon the left; a similar right or left-handed position of the ovary is described by Sommer[2] in *D. hepaticum*. The vitelline glands are two small compact lobes, lying close together, one on either side of the median line, just behind the ventral sucker. The uterus fills the circular body with its long, intricate coil; the contained eggs are thin-shelled and ellipsoidal, their axes measuring 25 μ and 17.5 μ. There is no cirrus, and neither male nor female genital opening is especially muscular. The male copulatory apparatus lies above the female apparatus and opens slightly in front of it into the common genital sinus.

The points most noteworthy in *D. patellare* are, externally, its pan shape, internally, its lack of pharynx and cirrus, the collection of its vitelline glands into two small compact lobes, the elongation of its excretory vesicle, and the great length of its uterus.

Histological.—The following histological points were studied:

1. Cuticle, subcuticula, and subcuticular cells.
2. The relation of the giant cells to nerves and muscles.
3. Flame-cells and capillaries of the excretory system.

1. *Cuticle, subcuticula, and subcuticular cells.* — The cuticle is a homogeneous, densely staining, single layer averaging 3 μ thick. In the genital openings and oesophagus it becomes much thicker (5 μ–6 μ) and shows a sort of stratification, staining alternately light and dark in layers parallel to the body surface. It encloses the nervous cores of the sensory papillae which are bulb-shaped and highly refractive, but I could find no "pore-canals," ducts, or traces of nuclei. Between cuticle and subcuticula, which are usually closely applied, a narrow space is

[2] SOMMER, F.: Zur Anatomie des Leberegels, *Zeit. f. wiss. Zool.*, Bd. xxxiv, 1880.

sometimes seen which is traversed by delicate fibrils connecting the two layers ; these fibrils are noted by Leuckart,[3] Brandes,[4] and Looss.[1 (a)] When the cuticle is torn away, the fibrils break at the subcuticula. Their distribution and appearance preclude the idea that they are inserting muscle fibrils or, as Brandes suggests, the ducts of glands ; it seems probable that they arise by the formation of vacuoles in the base of the cuticle and indicate a close connection between cuticle and subcuticula. The space traversed by these fibrils looks like a vacuolar layer and may correspond to the "vacuolar middle layer of the cuticle" described by some authors (Monticelli,[5] E. Walter,[6] Poirier[7]), their "basal layer of the cuticle" being what is here called the limiting membrane of the subcuticula. When cuticle meets epithelium it does not pass over it, under it, nor by transition into it, but ceases abruptly. There is in *D. patellare* no evidence that the cuticle is shed; the existence of well-differentiated nerve-endings in the cuticle seems, indeed, evidence to the contrary.

The tissue between the cuticle and the subcuticular cells, the subcuticula, has usually been described as undifferentiated. In *D. patellare* the outer part or limiting membrane is much more distinct from the inner reticular part in some specimens, where it is a thin, deeply staining sheet $\frac{1}{2}\ \mu$ thick, than in others, where it averages 1 μ thick, stains slightly, and passes almost imperceptibly into the subjacent reticulum. The limiting membrane forms sheaths for the inner surfaces of the suckers, where it contains fibrils which are probably elastic ; it receives the insertion of muscle fibrils, and passes by transition into the basement membrane of ducts into whose external openings it accompanies the cuticle. The fine meshwork of the intermuscular reticulum, which shows no cellular structure

[3] LEUCKART, R.: Die Parasiten des Menschen, Aufl. ii, 1886.

[4] BRANDES, A.: Zum feineren Bau der Trematoden, *Zeit. f. wiss. Zool.*, Bd. liii, 1892.

[5] MONTICELLI, FR. SAV.: Studii sui Trematodi endoparassiti, *Zool. Jahrbücher*, Suppl. III, 1893.

[6] WALTER, E.: Untersuchungen über den Bau der Trematoden, *Zeit. f. wiss. Zool.*, Bd. lxv, Heft 2, 1893.

[7] POIRIER, J.: Contribution à l'histoire des Trématodes, *Arch. d. Zool. expér. et gén.*, 2 sér., tome III, 1885.

and is often obscured by granules, is bounded internally with more or less distinctness by the subcuticular cells. The term subcuticula has been used interchangeably for the structures here called limiting membrane and intermuscular reticulum; thus Leuckart,[3] Braun,[8(a)] Monticelli,[5] E. Walter,[6] and Stafford[9] describe the limiting membrane as subcuticula, while Brandes,[4] Looss,[1(a)] and Braun[8(b)] describe as subcuticula the intermuscular reticulum. This fact together with the appearance of the subcuticula in *D. patellare* suggest that the limiting membrane is differentiated from the intermuscular reticulum, and that in some forms it may be noticeably developed, and not at all or only slightly in others. From the thickness and general appearance of the subcuticula it seems probable that it was once composed of cells, though no trace of these exists in the adult *D. patellare*.

There is hardly a point in the histology of Trematodes which needs study more than that of the subcuticular cells. The existence of a subcuticular cell layer, non-glandular, yet differing in appearance from the rest of the parenchyma, is maintained by Leuckart,[3] Ziegler,[10] Macé,[11] Looss,[1(a)(b)] E. Walter,[6] Schuberg,[12] and Goto[13] in Monogenea; the subcuticular cells are described as glandular by Brandes,[4] Monticelli,[5] Blumberg,[14] Poirier,[7] and Katheriner,[15] Brandes asserting and Monticelli denying that they form a continuous layer; while Saint-Rémy[16] denies the existence of any differentiated subcuticular cells in some forms.

[8] BRAUN, M.: Bronn's Klassen und Ordnungen des Thierreichs, Bd. Vermes. Trematoda. (a) Digenea. (b) Monogenea.

[9] STAFFORD, J.: Anatomical Structure of Aspidogaster conchicola, *Zool. Jahrbücher*, Bd. ix, Heft 3, 1896.

[10] ZIEGLER, H. E.: Bucephalus und Gasterostomum, *Zeit. f. wiss. Zool.*, Bd. xxxix, 1883.

[11] MACÉ, E.: Recherches anatomique sur la grande Douve du foie, Paris, 1881.

[12] SCHUBERG, A.: Zur Histologie der Trematoden, *Arbeit. a. d. zool.-zoot. Inst. Würzburgs*, Bd. x, 1895.

[13] GOTO, S.: Ectoparasitic Trematodes of Japan, *Journ. of Coll. of Science, Imp. Univ. of Japan*, 1894.

[14] BLUMBERG, C.: Ueber den Bau des Amphistoma conicum, *Inaugural-dissertation*, Dorpat, 1871.

[15] KATHERINER: Die Gattung Gyrodactylus, v. Nrdm, *Arbeit. a. d. zool.-zoot. Inst. Würzburgs*, Bd. x, 1895.

[16] SAINT-RÉMY, G.: Matériaux pour l'anatomie des Monocotylides, *Revue biologique du Nord d. l. France*, Ann. v, 1892.

In some specimens of *D. patellare*, the typical subcuticular cells form a continuous layer, most conspicuous in the neck ; in others they are modified here and there into glandlike cells; and in others modification has progressed so far, the glandlike cells degenerating, others losing their typical character, that the typical cells are confined to certain regions. In all the specimens studied these cells were found in their typical condition at the margins of the body, especially in the posterior end, and in the neck near the union of the body wall with that of the suckers. However the cytoplasm of these cells may be modified, they are (except in advanced stages of degeneration) distinguished from the rest of the parenchyma by their smaller, more deeply staining nuclei which are spherical, average 5 μ in diameter and have abundant chromatin, while those of the other cells are ellipsoidal, average 10 μ by 6 μ, and have one nucleolus and a small amount of chromatin. Nuclei transitional in character exist, however, between these two kinds. Some of the subcuticular nuclei are very small, 2 μ–4 μ, but these pass by transition into those of average size. Cells containing these characteristic nuclei are found beneath the subcuticula in all the specimens studied ; they are, as is noted by E. Walter,[6] coextensive with the cuticle and with that modification of it which lines Laurer's duct and the excretory vesicle, ceasing abruptly with the cuticle. The cells described here and by most writers as typical subcuticular cells are small, polyhedral or rounded, and have a finely granular, densely staining cytoplasm, while the cells of the inner parenchyma are large with a vacuolar cytoplasm, but in this Distomum the former pass by transition into the latter. The glandlike modifications of these cells have usually flask-shaped bodies, whose necks are directed toward the cuticle but cannot be traced beyond the muscular layer, and a finely granular cytoplasm containing large, highly refractive granules. These glandlike cells degenerate ; their nuclei shrink, their granules aggregate into irregular, deeply staining masses, lose their refractive power, and finally disintegrate ; the cells become finely vacuolar and neighboring ones may fuse forming larger vacuolar masses which finally lose all trace of a cellular structure. The

typical cells among which these degenerating ones lie usually become somewhat vacuolar and their cell boundaries may partly break down so that they form a coarse reticulum in which, however, examination always shows typical subcuticular nuclei.

The tissues are well preserved, so that these changes in the subcuticular cells can hardly be considered abnormal; since, however, the assumption of a glandlike form is not accompanied by any apparent change in cuticle or subcuticula and is followed by degeneration, it seems probable that the change is due to age, and the appearance of the other subcuticular cells confirms this idea. One recalls in this connection the suggestion of Looss [1(a)] that the presence of the subcuticular cells as a definite layer may depend upon the age of the specimen studied, and the facts found here suggest that possibly their glandular or non-glandular appearance may depend upon a similar condition. It is difficult to compare the glandlike cells found here with the glandular subcuticular cells noted by others, for descriptions and figures are seldom definite enough on this point; but it seems possible that they correspond to those noted by Blumberg,[14] to some at least of those subcuticular cells described by Monticelli as skin glands (see Looss [1(a)]), and to the glandlike cells described by Poirier.[7] No degeneration stages such as exist in this form are described by others. The transition of the subcuticular cells into the subjacent parenchyma shows that they must be considered peripheral traces of primitive parenchyma as Leuckart, Macé, Ziegler, Schuberg, and Goto suggest; while their coextensiveness with the cuticle and their ability, in some species at least, to assume a glandlike form (although in *D. patellare* this does not seem to be functional in the adult) indicate that, as Looss and E. Walter suggest and Brandes and Blumberg affirm, they may have a glandular function in some way connected with the cuticle.

On the whole, the facts found in connection with the cuticle, subcuticula, and subcuticular cells of *D. patellare* seem to me to support the view that the cuticle is not a true cuticle, a modified epithelium, or a basal membrane, but a pseudo-cuticle formed when the larval epithelium was lost. It seems prob-

able that this pseudo-cuticle was formed, not by the subcuticular cells, but by cells of similar origin of which the subcuticular cells and possibly the subcuticula are traces.

2. *The Relation of the Giant-cells to Nerves and Muscles.* — Monticelli[5] identifies the giant-cells with ganglion cells, showing that they are in some parts of the body directly connected with nerves, and his work is confirmed by Schuberg,[12] although the nervous connection of giant-cells in the posterior part of the body remains undetermined. Blochmann and Bettendorff,[17] however, noting a close connection between giant-cells and muscle-fibers, homologize them with the myoblasts found in Cestodes by Blochmann.[18] Zernecke[19] distinguishes the myoblasts of Cestodes from the ganglion and sense-cells ; the former are muscle-formers, one process of each of which has a secondary nervous connection, the latter generate the nervous system, in whose central or peripheral parts they lie. Finding the giant-cells favorable in *D. patellare*, I have studied their relation to nerves and muscles.

The histology of the muscles and nerves must be briefly given. Each muscle is a bundle of parallel longitudinal fibrils, around which I can detect no sheath. These fibrils insert separately at the end of the fiber into the limiting membrane. The large muscles are often hollow. The dorso-ventral and radial muscles are simple fibers, but the others branch and their branches anastomose, so that each muscle becomes a complex system of parallel anastomosing fibers. The longitudinal and diagonal muscles are especially long and complex. I could not find muscle nuclei.

The cerebral ganglia and nerve-trunks are composed of a finely reticular substance, in whose meshes granules exist; no nuclei are found in it, no cells are connected with it except the giant-cells, and it has no sheath. The ventral nerves, which can be followed with an oil-immersion lens for some distance,

[17] BLOCHMANN UND BETTENDORFF : Ueber Muskulatur und Sinneszellen der Trematoden, *Biolog. Centralbl.*, Bd. xv, No. 6, 1895.

[18] BLOCHMANN, F.: Ueber freie Nervenendigungen und Sinneszellen der Bandwürmern, *Biolog. Centralbl.*, Bd. xv, No. 1, 1895.

[19] ZERNECKE, E.: Ueber den Nervensystem bei Bandwürmern, *Zool. Jahrbücher*, 1896.

break up finally just within the dermal musculature into branches composed of delicate anastomosing fibrils; these branches do not disintegrate into separate fibrils whose endings can be traced, but spread out, forming very delicate plexuses. The ventral nerves were traced farthest, but all the others pass finally to the body wall, and probably end in the same way. The sensory papillae (Fig. 2) resemble those described by Blochmann and Bettendorff[17] as existing over the body and in the suckers of Trematode cercaria. Each granular nervous bulb has, in the body papillae, a dense fibrillar core which terminates at the end of a small prominence at the apex of the papilla. The rest of the bulb has often a fibrillar appearance, the granules being arranged in longitudinal rows, but in a few cases the fine granules are replaced by a few large, deeply staining ones. From the inner end of the bulb a fiber which looks like a continuation of the fibrillar core passes down into the intermuscular reticulum, and this fiber may, by careful focussing upon tangential sections of the body wall, be followed until, just outside of the dermal muscles, it breaks up into delicate anastomosing fibrils which resemble those into which the ventral nerves disintegrate. No cells, except in a few cases the giant-cells, were found connected with the sensory papillae; no sense-cells like those figured by Blochmann and Bettendorff were found.

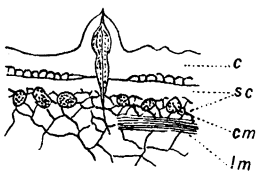

FIG. 2. — Sensory papilla from the body. *c*, cuticle; *sc*, sub-cuticula; *cm*, circular muscle; *lm*, longitudinal muscle.

Small groups of large, deeply staining granules, like those described in some of the sensory papillae, are scattered irregularly in the subcuticula. The granules are solid-looking, and those of a group usually lie close together in a straight line. As they were first seen lying parallel to muscles, close to them, and sometimes between the muscle fibrils, I thought for a time that they might be traces of muscle nuclei, but I afterwards found that they lie beneath the muscles and independent of them. They may be a form of nerve-ending simpler than the sensory papilla.

The body of the bi- or multipolar giant-cell varies from 20 μ to 40 μ in diameter, the processes from 30 μ to 50 μ in length. The ovoid or ellipsoidal nucleus is distinguished by its size (12 μ by 9 μ) and by its one large nucleolus from all the other nuclei of the body save those of the excretory cells, which it closely resembles. The cytoplasm is a fine reticulum whose meshes may elongate so that it becomes fibrillar-looking; usually it looks reticular around the nucleus and more fibrillar toward the cell-periphery and in the processes. Fine granules are scattered in the meshes of the cytoplasm and occasionally a number of large, deeply staining granules occur, like those found in some of the sensory papillae.

Most of the giant-cells are found near the body wall, but they also occur near the cerebral ganglia and nerve-trunks, are scattered through the suckers, and very fine specimens lie upon or near the extrinsic muscles of the ventral sucker. The size of the cells and the winding of their processes prevented me from tracing the distribution of all the processes of any one cell. Processes of these cells may pass (*a*) into the nerve-trunks or ganglia, (*b*) to muscle-fibers, (*c*) to the sensory papillae, (*d*) to the body wall where they spread out as the endings of the ventral nerves do, in a plexus of delicate, anastomosing fibrils.

(*a*) I can confirm on this point the work of Monticelli and Schuberg.

(*b*) Many of the giant-cells are connected by at least one of their processes with a muscle. Most of the processes of the multipolar cells lying near the base of the ventral sucker were traced to its extrinsic muscles; but, contrary to what Blochmann and Bettendorff found, fibers belonging to different anastomosing systems may be supplied with processes from the same giant-cell. This indicates that these cells are not myoblasts. The processes passing to the muscles break up into varicose anastomosing fibrils which can be traced for some distance along the surface of the muscle, developing here and there delicate thickened endings which are closely opposed to the muscle fibrils (Fig. 3).

(*c*) The fibrils of a process passing to a sensory papilla converge until they apparently fuse to form the fibril entering its

base. The different parts of the papillae may be brought into view by focussing on tangential sections of the body wall.

(*d*) The processes which spread out into plexuses cannot be followed far, for after the outline of the process is lost, it is easy to deceive oneself. The same is true in the case of the ultimate fibrils of the ventral nerve and those passing from the sensory papillae. I cannot, therefore, assert that a continuous subcuticular plexus is formed by processes of the giant-cells and ultimate branches of the nerve-trunks, yet the facts suggest this. I hope to test fresh material on this point with special methods. Blochmann and Zernecke find the peripheral plexus in Cestodes within the subcuticular cells, while here the indications are that it lies outside of them, within the dermal muscles. Blochmann and Bettendorff state that a nervous plexus exists within the subcuticular cells in Trematodes, but their preliminary paper gives no satisfactory description or figures of it.

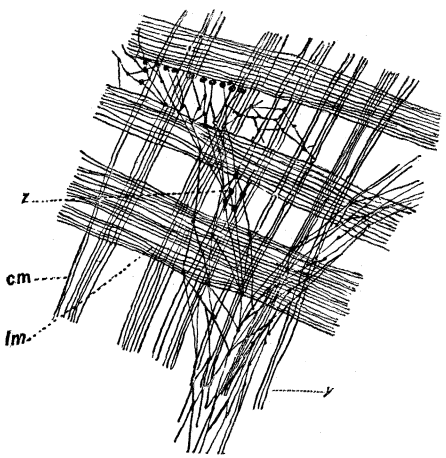

FIG. 3. — Tangential section of body wall. *cm*, circular muscle; *lm*, longitudinal muscle; *y*, process of giant-cell; *z*, process innervating muscle.

These facts seem to indicate that the giant-cells are not myoblasts but true ganglion cells, some of which have retained a primitive character so that they lie at the periphery and innervate both muscles and sense-organs, either directly or through a peripheral plexus. The nervous system is simpler here than in Cestodes, for one cell, the giant-cell, does the work which in Cestodes is assigned to three, — sense-cell, ganglion-cell proper, and myoblast. This slight differentiation in the sensory-motor system reminds one of the instances mentioned by Zernecke where he found in Cestodes a direct connection between sense-cell and myoblast.

3. *Flame-cells and Capillaries of the Excretory System.* — I can confirm the work of Schuberg [12] on these points. The excretory capillaries have a cellular wall of their own, containing large, though widely scattered, nuclei (Fig. 4), and are terminated by closed flame-cells (Fig. 5). No permanent lacunar spaces exist in the parenchyma, the excretory matter being passed to and through the flame-cells by osmosis. The processes of the protoplasmic part of the flame-cell extend between the parenchyma cells. Deeply staining (muscular?) thickenings are found in the capillary walls. There is a marked resemblance between the nuclei of the giant-cells and those of the capillaries and flame-cells, — a resemblance which is, perhaps, partly responsible for the observation of excretory capillaries in the suckers, and for other confusion which has existed between the giant-cells, and cells of the excretory system (see G. Walter,[20] Villot,[21] Macé,[11] Braun [8(a)] upon the peripheral giant-cells, and Wright and Macallum [22] upon the "renal" cells of Sphyranura).

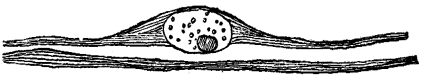

FIG. 4. — Part of excretory capillary, showing nucleus.

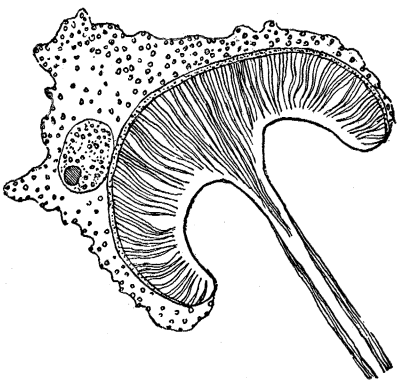

FIG. 5. — Flame-cell.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
April 20, 1897.

[20] WALTER G.: Beiträge zur Anatomie und Histologie einzelner Trematoden, *Arch. f. Naturgesch*, Bd. i, 1858.

[21] VILLOT, A.: Organization et développement de quelques Trematodes endoparasites marins, *Ann. des Sci. Nat.*, tome viii, 1878.

[22] WRIGHT AND MACALLUM: Sphyranura Osteri, *Journ. of Morph*, vol. i, 1887.